vit D_2-induced hypomagnesemia would appear to be a redistribution of this cation from the extracellular phase to some tissue, presumably bone, independent of any specific influence on protein-binding of magnesium. The lack of difference between the muscle and carcass levels of magnesium in the two groups is compatible with this suggestion.

The slight but significantly depressed level of serum potassium of the vit D_2-treated group is interesting in view of the known adverse effect of this vitamin on renal conservation of potassium(1), but, unfortunately, urine potassium excretion was not determined.

Summary. The hypomagnesemia consequent to administration of large doses of vit D_2 to rats was studied. Two groups of animals were pair-fed a magnesium-deficient diet, and one was then given injections of vit D_2. Hypomagnesemia resulted in the vit D_2-treated group. During the period of vitamin administration there was no significant difference between the urinary or fecal magnesium excretion of the 2 groups. Nor were the differences significant between the levels of magnesium in the total carcass and muscle. The level of serum proteins also was not different in the 2 groups. It is suggested that the hypomagnesemia is due to a redistribution of magnesium from the extracellular phase to some other depot, which may well be bone. One mechanism might involve a reduced binding of magnesium by serum proteins due to some specific effect of vit D_2 (or perhaps to the resultant hypercalcemia). An alternative hypothesis is that vit D_2 has some other specific influence that promotes a deposition of magnesium in bone or some other depot.

1. Hanna, S., Metabolism, 1961, v10, 735.
2. Richardson, J. A., Huffines, W. D., Welt, L. G., *ibid.*, 1963, v12, 560.
3. Whang, R., Welt, L. G., J. Clin. Invest., 1963, v42, 305.
4. Hollander, W., Jr., Winters, R. W., Williams, T. F., Bradley, J., Oliver, J., Welt, L. G., Am. J. Physiol., 1957, v189, 557.
5. Barry, K. G., McLaurin, A. W., Parnell, B. L., J. Lab. Clin. Med., 1960, v55, 803.
6. MacIntyre, I., Adv. Clin. Chem., 1961, v4, 1.

Received October 19, 1964. P.S.E.B.M., 1965, v118.

Enzymatic Degradation of Pentaerythritol Tetranitrate by Human Blood. (29892)

FREDERICK J. DICARLO, JAMES M. HARTIGAN, JR., AND GEORGE E. PHILLIPS
(Introduced by William M. Govier)
Biochemistry Department, Warner-Lambert Research Institute, Morris Plains, N. J.

After finding that the administration of pentaerythritol tetranitrate (PETN) leads to excretion of pentaerythritol (PE) by mice (1,2) and rats(3), it became of interest to learn whether blood alone possesses the capacity to degrade PETN. This point was investigated *in vitro* with human blood. Also evaluated were human blood plasma and erythrocytes. The study was conducted by incubating C^{14}-PETN with blood, plasma and red blood cells, sampling at various time intervals, and analyzing the C^{14} compounds by thin layer chromatography and radio-scanning(4). C^{14}-PETN in phosphate buffer was employed as a control system.

Materials and methods. C^{14}-PETN, labeled at carbon atoms 1 and 2, was synthesized from acetaldehyde *via* PE. The product (m. pt., 140-141.5°) was mixed with 7 parts by weight of C.P. lactose in order to minimize the explosion hazard. The activity of the lactose-C^{14}-PETN mixture was 0.59 millicurie/g.

Blood plasma prepared from human citrated whole blood was used without dilution. After repetitive washing with physiological saline, red cells from the same source were resuspended in saline to the original blood volume.

Aliquots (15.0 ml) of 0.4 M phosphate

TABLE I. Degradation of C^{14}-PETN by Human Blood.

Medium	Time (hr)	Percent radioactivity identified*			
		PETN	PE-Trinitrate	PE-Dinitrate	PE-Mononitrate + PE
Phosphate buffer	1	100	.0	.0	.0
" "	2	100	.0	.0	.0
" "	4	100	.0	.0	.0
" "	6	100	.0	.0	.0
" "	24	91.8	8.2	.0	.0
Whole blood	1	36.9	53.2	9.9	.0
" "	2	31.9	56.3	7.7	4.1
" "	4	25.9	63.7	8.2	2.2
" "	6	17.7	64.5	11.3	6.5
" "	24	.0	27.4	64.4	8.2
Blood plasma	1	82.2	17.8	.0	.0
" "	2	80.0	20.0	.0	.0
" "	4	70.0	25.4	4.6	.0
" "	6	58.6	39.0	2.4	.0
" "	24	29.0	57.6	10.9	2.5
RBC suspension	1	37.8	55.5	6.7	.0
" "	2	37.1	46.9	8.3	7.7
" "	4	25.0	54.0	13.3	7.7
" "	6	16.0	67.7	13.0	2.3
" "	24	.0	32.7	60.6	6.7

* By thin layer chromatography in solvent A, the Rf values found for reference preparations were PETN (.70), PE-trinitrate (.50), PE-dinitrate (.20), PE-mononitrate (.05), and PE (.00). Corresponding Rf values in solvent B were: .85, .80, .70, .35, and .00.

buffer (pH 7.4), citrated whole blood, plasma and erythrocyte suspension were transferred to 50 ml glass-stoppered Erlenmeyer flasks. To each flask was added 1.0 ml of ether containing 100 μg of C^{14}-PETN. The flasks were set into a water bath at 37°, kept uncovered for the first hour in order to allow the ether to evaporate, and then stoppered. At specified intervals, 2.0 ml aliquots were withdrawn from each vessel and extracted twice with 4.0 ml volumes of ether. The extracts were reduced to dryness by an air stream at room temperature. Each residue was dissolved in 1.0 ml of ether and 500 μl aliquots were spotted for thin layer chromatography on glass plates (2″ × 8″) coated with silica gel G. The solvent systems employed were: A. toluene-ethyl acetate (1:1, v/v) and B. ethyl acetate saturated with water.

The chromatograms were scanned for radioactivity with a Nuclear Chicago Actigraph II (model 4502) equipped with a thin layer chromatography adaptor (model 1039). The area under each curve was determined with a Keuffel and Esser compensating polar planimeter.

Results. Table I shows the course of PETN degradation in the 4 tested media as determined with solvent A. Since this solvent system did not adequately resolve PE and PE-mononitrate, efforts were made to employ solvent B for this purpose, but the quantities of these compounds were too low for precise determination. Nevertheless, it is clear that blood, blood plasma and erythrocytes degrade PETN extensively, whereas, the only conversion effected by phosphate buffer was the hydrolysis of about 8% of the PETN to PE-trinitrate after 24 hours.

The erythrocytes suspended in saline to original blood volume were about as effective as whole blood. Blood plasma, on the other hand, was less effective than whole blood although plasma was employed without dilution.

Discussion. A comparison of the conversions of PETN effected by buffer and by blood indicates that PETN is degraded enzymatically by blood. It is also clear that the erythrocytes contain the enzyme system.

An earlier study of the hydrolysis of PETN by acid showed it to be hydrolyzed at approximately the same rate as the tri-, di-, and mononitrates of PE(4). The enzymatic breakdown of PETN does not follow this course. Instead, it is evident that PETN is converted into PE-trinitrate faster than the

trinitrate is degraded to PE-dinitrate, and that the next step proceeds still more slowly.

Crandall(5) reported that nitroglycerol (NG) and erythritol tetranitrate (ETN) are attacked enzymatically by dog blood erythrocytes. Yagoda and von Oettingen(6) extended the study and showed that NG, ETN and PETN release inorganic nitrite during incubation with dog blood. These investigators did not identify the resultant organic products, but isolated unchanged compounds (5) and measured inorganic nitrite. Heppel and Hilmoe(7) demonstrated that NG and ETN oxidize glutathione and that the reaction is catalyzed by an enzyme present in liver and other tissues. The reaction products identified *in vitro* and *in vivo* were oxidized glutathione and inorganic nitrite. Needleman and Krantz(8) further purified NG reductase(9) from hog liver and heart, and employed thin layer chromatography to follow the course of the enzymatic degradation of NG. They reported the formation of 2 unidentified organic products in addition to inorganic nitrite and traces of aldehyde and ketone in the reaction mixture. No glycerol was detected.

Since NG reductase is not specific for NG, but is known to attack ETN, it seems probable that the enzyme is responsible also for initiating the degradation of PETN. Our identification of PE-mono-, di-, and trinitrates as enzymatic conversion products from PETN implies that the unidentified products of NG degradation(8) are mono- and/or dinitrates of glycerol. Since glycerol is not symmetrical like PE, it can yield 2 mononitrates and 2 dinitrates. One may speculate also that the traces of aldehyde and ketone (8) may be artifacts derived from glutathione.

Needleman and Krantz(8) considered that enzymatic reduction is too slow to account for the rapid pharmacological effect of NG and concluded that NG itself is the active anti-anginal agent. This hypothesis may account for the longer duration of action of PETN which apparently is a more resistant substrate than NG(6). The same line of reasoning and the greater resistance of other PE nitrates to enzymatic attack imply that these nitrates may contribute materially to anginal prophylaxis ascribed to PETN.

Summary. In vitro, human blood enzymatically degrades PETN stepwise to yield PE-trinitrate, PE-dinitrate, PE-mononitrate, and possibly PE. The enzyme system appears to be concentrated in the erythrocytes.

The authors are indebted to Mr. Edward Merrill for C^{14}-PETN and to Dr. Eric Goldschmidt for valuable consultation.

1. DiCarlo, F. J., Hartigan, J. M., Jr., Phillips, G. E., Life Sciences, 1964, v3, 563.

2. DiCarlo, F. J., Hartigan, J. M., Jr., Coutinho, C. B., Phillips, G. E., Proc. Soc. Exp. Biol. and Med., in press.

3. DiCarlo, F. J., Coutinho, C. B., Phillips, G. E., to be published.

4. DiCarlo, F. J., Hartigan, J. M., Jr., Phillips, G. E., Anal. Chem., 1964, v36, 2301.

5. Crandall, L. A., Jr., J. Pharmacol. Exp. Therap., 1933, v48, 127.

6. Yagoda, H., von Oettingen, W. F., U.S. Pub. Health Bull., 1944, No. 282, 8.

7. Heppel, L. A., Hilmoe, R. J., J. Biol. Chem., 1950, v183, 129.

8. Needleman, P., Krantz, J. C., Jr., Fed. Proc., 1964, v23, 178.

9. Mapson, L. W., Glutathione, Crook, E. M., Ed., Cambridge Univ. Press, London, 1959, 32.

Received October 23, 1964. P.S.E.B.M., 1965, v118.